Cells into Organs

THE FORCES THAT SHAPE THE EMBRYO

SECOND EDITION

John Philip Trinkaus

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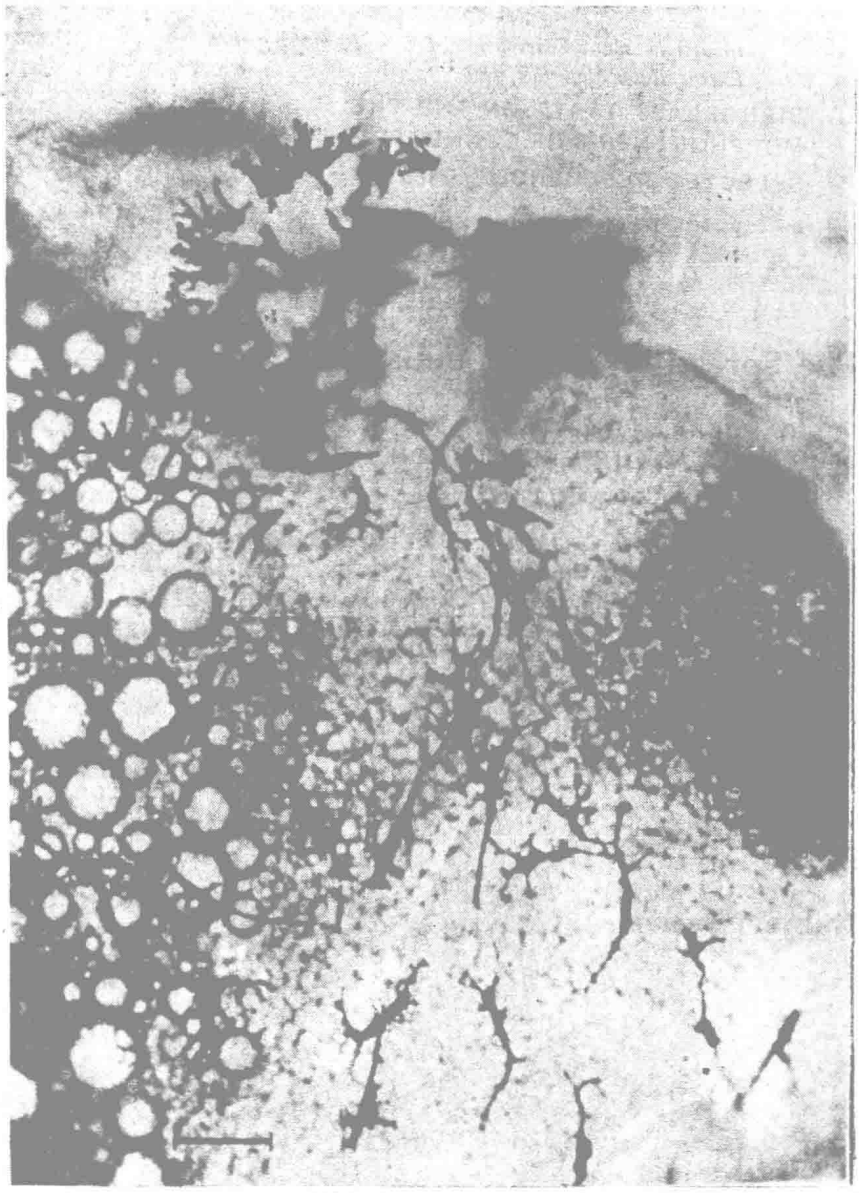

Frontispiece. A montage showing dendritic melanocytes in the yolk sac of an embryo of the teleost *Blennius pholis* moving toward the pectoral fin bud (in the upper left quadrant). Note the predominantly dorso-ventral orientation of the migrating melanocytes. At completion of this directional cell movement, each melanocyte penetrates the pectoral fin bud, moves within it, and becomes aligned with other melanocytes to form a distinctive radial pattern, with the dendritic protrusions of each cell extending distally. Part of the otic vesicle, anterior to the pectoral fin bud, is evident in the upper right quadrant. Scale bar, 80 μm. For further description, see pp. 330–334 of Chapter 11. (Trinkaus, unpublished.)

Preface

FIRST EDITION

And the end of all our exploring
Will be to arrive where we started
And know the place for the first time.

T.S. Eliot, *Little Gidding**

This book is concerned with the origins and changes in form of tissues and organs. Although recognition of the importance of these phenomena is as old as embryology itself, sustained investigation of them is a modern development. Doubtless a major reason for this avoidance of problems so close to the heart of embryology was what Professor Ross G. Harrison liked to call the "gold rush" toward the analysis of embryonic induction, which for so long diverted attention from other problems of development. But another reason surely was the rather primitive state of our knowledge of cell surface behavior. In spite of pioneering studies by Harrison, Holtfreter, Lewis, Weiss, and others through the years, detailed knowledge of how cells contact each other, how they move about, and how their individual activities are converted into the collective movement of cell sheets was until recently largely lacking. Now, however, this information is becoming available, due in substantial degree to improved methods of culturing cells and the invention of a number of techniques that permit closer study of cell contact phenomena. For some time the study of normal tissue cells and cancer cells in culture and the study of mechanisms of morphogenetic cell movements in the embryo were largely separate, in detriment particularly to the latter. Now, however, the fields have converged. This is of course to their mutual profit, and progress recently has perceptibly acceler-

*From "Little Gidding" in *Four Quartets*, copyright 1943 by T. S. Eliot; renewed 1971 by Esme Valerie Eliot. Reprinted by permission of Harcourt Brace Jovanovich, Inc.

ated, so much so in fact that study of the cell surface in all of its aspects is rapidly becoming one of the most active areas of cell and developmental biology.

The purpose of this book is to try to pull together some of this work on cell contacts and movements, particularly as they relate to changes in the shape of multicellular systems during development. This is where my own interests lie, and it is in this area and in the area of cancer cell movement that I believe some of the most important problems ultimately lie. My intent has been to describe the current situation as it appears to me and to raise questions, rather than survey the field and record all that has been done. Since I have not striven for completeness, much has been left out or given limited consideration. Rather less attention, for example, has been devoted to what might be termed the molecular features of cell contacts than might be thought desirable. This is in part because at present this is an almost entirely speculative area with little substantial that has been really well-established and in part because the subject has been reviewed recently in depth in books by A.S.G. Curtis and L. Weiss. It is true, of course, that speculations of this sort can be useful, in that they may suggest what to look for in the laboratory. But it is also true that oftentimes questions must first be answered on a cell and fine-structural level before we are in a position to pose biologically meaningful questions on a physicochemical level. In any case, my abridged treatment of the subject certainly does not reflect conviction on my part that the solutions to problems of cell movements lie elsewhere. On the contrary, along with everyone else I believe that it is precisely in the molecular structure of the cell membrane, in the dependence of this structure on the genome or indeed in its independence of the genome, and in its relations to various substrata during cell contact and movement that solutions to the problems posed by tissue and organ shape changes are ultimately to be found.

Another area that has received relatively little attention in this book is plant morphogenesis. Much less space is given to plant cells than to animal cells. There is good reason for this. The higher plants do not utilize cell movements as a morphogenetic mechanism (and in this lies what is probably the most important difference between plant and animal morphogenesis). In certain of the lower plants, however, in particular the cellular slime molds, cell movements have supreme significance in morphogenesis. I have therefore dealt with them at some length.

The book is thus more an essay than a survey—an essay on some of the major problems that confront cell and developmental biologists when they think about cell contacts, cell movements, and morphogenesis. Hopefully, it will alert the reader to some of the areas that most desperately need attention and stimulate research activity. The book will have succeeded if it accelerates its already burgeoning obsolescence.

In keeping with the decision not to seek completeness, no consistent effort has been made to give detailed credit for work discussed. For this

I ask the indulgence of my colleagues. However, since the purpose of the book is to stimulate interest in morphogenetic movements and cell contact phenomena, a means must be provided for moving beyond the text and finding the original works on which the discussions are based. To this end, a list of "selected references" is appended to each chapter. These titles have been selected partly because they describe work that I consider important and partly because they are recent and contain useful bibliographies. All investigations described in the text can be traced readily, either directly or indirectly, by means of these reference lists. A few works of a more comprehensive nature have been listed at the beginning to introduce the reader to subjects not covered or given only nodding attention in the present work. In a word, even though the reference lists are by no means complete, they can be used as an entry into the literature and a guide as to where to find it.

This volume was written in a small village in the south of France, where distracted only by the sun, the sea, and an occasional game of pétanque I was free to read, reflect, and write at length on problems close to my heart. I am indebted to my wife who gave up untold hours in the sun, with hardly a complaint, to act as my typist. I am also grateful to my colleagues Adam Curtis, Clement Markert, and James Weston, all of whom read the manuscript and made many suggestions, some of which I adopted. I am indebted to Linda Krulikowski and Dolores Slaughter for aid with the references, the figures, and the index. Finally, I wish to express my gratitude to students and colleagues at Yale University and at Woods Hole for many profitable discussions of these problems.

My research through the years and the writing of this book have been supported by grants from the National Science Foundation.

Hurlevent
Le Castellet (Var), France
August, 1968

J.P. TRINKAUS

Preface

SECOND EDITION

Although this volume is billed as the Second Revised Edition of *Cells into Organs: The Forces That Shape the Embryo*, the revision is so extensive that it is almost a new book. Fifteen years ago, when the first edition went to press, I noted that the field of cell motility was "rapidly becoming one of the most active areas of cell and developmental biology." Now, this prediction has long since been realized, with accompanying advances in our understanding both of problems posed in the first edition and others that had not yet been postulated—in my book or anywhere else. In consequence, the task confronting me, as I began to contemplate revision, was substantially greater than the one of writing my little book in the first place. Moreover, as I progressed in the revision, the field progressed as well. Thus, the revision itself required constant revision so that the end-product might have some semblance to the state of the field.

In addition to this effort to update both substance and concept generally throughout the text, I have made some other important changes. Full chapters have been added on the molecular structure of the plasma membrane and the spread of cancer—subjects that were hardly touched on in the first edition. My treatment of the movement of tissue cells in culture, in particular fibroblasts, has been greatly expanded, in recognition of genuine advances in our understanding of the movement and contact relations of these important cells during the last decade. And I have totally reorganized my discussion of morphogenetic movements, the dominant theme of the book. My approach this time is frankly

horizontal—movement by movement—rather than hesitatingly vertical—organism by organism (as in the first edition). I might say that I embarked on reorganization of this part of the book with some zest, for it reflected my conviction that there has been significant progress in our understanding of cell movements within organisms in recent years. The similarities in the way cells move in different embryos seem now to be more impressive than the differences. There no doubt will be some objection to my organization of this material. Conceivably, another organization would be more useful. But, then, since the subject is really a continuum, breaking it up into any categories or chapters is bound to be artificial to some degree, and therefore faulty (like the various ways we organize our universities into departments). I have tried to reduce this artificiality by deliberately introducing a certain amount of overlap and by abundant cross-references in the text. But, of course, the best cross-references will be in the mind of the reader.

Otherwise, my overall purpose and approach in composing the Second Edition have been the same: to pull together what I find to be some of the more interesting results of research on the motility of tissue cells, particularly as they relate to invasiveness and changes in cell shape during morphogenesis and the spread of cancer, and to raise questions. Like the First Edition, this book is really an extended essay, whose principal aim is to stimulate research, especially on the part of biologists not currently working in the field, rather than to present a survey of its current state. This book was emphatically not written for colleagues working on cell motility (although parts of it may be useful to some of them). It is intended primarily for advanced students and researchers in other fields of biology, who know some embryology and some cell biology and wish to gain a kind of overview of some of the major problems of the field of cell motility. A book that treats this subject matter, and where the emphasis is mainly on the movements of tissue cells within organisms, may be of particular value to them.

Because my emphasis is as much on what remains to be done as on what has already been done, the text may often seem to project a negative tone. This is not intended. Quite the reverse. If we are to build in the future, we must understand where we stand in the present. This entails knowing what we do not know as well as what we do, and, where possible, how we may go about reducing the unknown. I have tried to do this by lacing the text with suggestions.

Since the current interest in tissue cell motility is relatively recent in origin and the field is replete with unsolved problems and tantalizing mysteries, it is not begging for hypotheses. This is a sign of youth, interest, and vigor; but there are subtle dangers. Richard Campbell has put it well: . . . many investigators . . . have been led into pitfalls . . . by presuming the existence of a particular developmental mechanism and then gathering evidence for it. Since it is usually possible to obtain data consistent with almost any proposal, there is a danger to working solely

upon a particular hypotheses." Keeping this in mind, I have striven in these pages to achieve a balanced treatment, taking into account all current major hypotheses where applicable.

Some will find that there is rather less emphasis in this book on research at the molecular level than they might desire. There are two reasons for this. Much of the molecular literature is well-summarized in a number of reviews elsewhere. The main reason, however, is that I have tried to limit myself to work that has actually brought us closer to an understanding of how *whole* cells move. More often than not, this has meant more concentration on discoveries at the cell level and at the level of cellular organelles than at the molecular level. Although we are all confident that we will ultimately understand cell movement in terms of molecules of the cell surface and the cytoskeleton, we are as yet a long way from such understanding. Major stumbling blocks have been our incomplete knowledge of the dynamic morphology of moving cells, and insufficient attention given to cellular form and function at the moment of the molecular analysis. Molecular studies that are not based on detailed attention to what the cell is doing at that time are likely to be sterile. Although our appreciation of cell motility at the morphological level is still incomplete, nevertheless there has been some important progress in recent years and, with this, a firm basis is being laid for meaningful investigation at the molecular level. In the meantime, it is well to be reminded that "molecular" does not necessarily mean "analytical" nor, by contrast, does "cellular" or "histological" necessarily imply "descriptive." Sound descriptive and analytical studies are obviously required at both (and all) levels of organization. Snobbish references to certain morphological studies as "merely descriptive" only serve to reveal the naivete of the authors and, if insisted upon, could impede advance of the field. After all, running a gel is also descriptive, but no one questions its utility as a means of learning what molecules are there.

Now a word about terminology. In any area of science that is still in a rudimentary state, as is the study of cell motility, there are bound to be the problems of terminology. Sometimes these terminological matters are merely annoying, as when new proteins are given a name that implies a function not yet demonstrated; but often, they interfere with communication. I have made a serious effort throughout the text to be terminologically responsible, but am surely not beyond criticism. When tussling with the names of things in a search for precision of expression, I am afraid many of us find ourselves all too often following Lewis Carroll: "When I use a word," Humpty Dumpty said in a rather scornful tone, "it means just what I choose it to mean—neither more nor less."

Finally, in keeping with the style of the first edition of this book and with the decision not to seek completeness, I have made no consistent effort to cite references and give detailed credit. For this I again ask the

indulgence of my colleagues. As previously, however, I do suggest some general references (see page xxv) and append a list of references to each chapter; these should be adequate for those who wish to push onward. I am anxious that anyone wishing to cite anything I have discussed be forced to the source. If it is not found immediately in the "Selected References," nor in the figure captions, it can acertainly be tracked down by means of them. Incidentally, the reader will note that I give some attention to the older literature. This represents, in part, a feeble effort to combat a distressing situation—the current proclivity to ignore the past, as if science were founded yesterday. Much that is reported nowadays as new has really been known for a long time and would not be so reported had the author read the literature. Good science is good science, whenever and wherever.

This book was put together in bits and pieces at various times and in various places during the last several years: in the village of Le Castellet and the vineyards of La Cadière d'Azur in the south of France, in the rarefied atmosphere of the laboratory of S.J. Singer in La Jolla, at the Station Biologique de Roscoff, at the Kewalo Marine Laboratory in Honolulu, at the Marine Biological Laboratory in Woods Hole, at my home in the woods of Guilford, Connecticut, and, finally and mainly this last year, at Yale University. I am indebted to Jon Singer and John Arnold for their hospitality, and to Yale University for its civilized system of academic leave.

One of the enduring pleasures that accompanies a scientific effort as extensive as the composition of this volume stems from a basic feature of all scientific activity—its collectivity. Although I produced the manuscript and take responsibility for all contained therein, much of the thinking is collectively derived. Many of my colleagues and students have contributed. Since they are too numerous for everyone to be mentioned, let me simply express my gratitude to all who gave of their time and thinking, whether personally or through their publications. Certain individuals among them, however, deserve special mention. Outside of myself, no one contributed more to the substance of the manuscript than my colleague Albert Harris. He read the entire penultimate version, thought about it, and (typically) criticized relentlessly where he thought it necessary. Although (naturally) I did not adopt all of his suggestions, the manuscript and my reflections on these problems benefitted greatly. Others who also deserve special thanks are Richard Campbell and Raymond Keller, who also read the penultimate version and improved it in important ways. Finally, I am indebted to P.B. Armstrong, L.L. Englander, G. Gerisch, S.A. Roth and M.S. Shure, who contributed useful criticisms at various junctures along the way.

Even though he has not been with us now for some years, I wish to express a long-standing indebtedness to Professor Ross Granville Harrison. I had the privilege, along with anyone else who wished, to lunch with him almost daily in the attic of the Osborn-Zoological Laboratory

during my early years at Yale University. He was such a wise, cultured, generous gentlemen—a genuine inspiration.

As I ponder the long preparation of this book, it is hard to imagine how I could have gotten this far without the aid of my wife Madeleine and her trusty word processor. I am indebted to her for her skill, her patience with my endless changes, her cogent stylistic suggestions and her aid with the references, the illustrations, and the index. Lastly, I wish to express my appreciation to Tom Aloisi of Prentice-Hall, Inc., who nursed my manuscript, figures, references, and all, through the several stages that have led to its successful emergence as an intact book

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New Haven,
Connecticut
January, 1984

J.P. TRINKAUS

General References

ABERCROMBIE, M. 1977. Concepts in morphogenesis. Proc. R. Soc. Lond. B 199: 337-344.

ALLEN, R.D., and N. KAMIYA [eds.]. 1964. Primitive motile systems. Academic Press, Inc., New York. 642 p.

BELLAIRS, R., A.S.G. CURTIS, and G. DUNN [eds.]. 1982. Cell behaviour. Cambridge Univ. Press, Cambridge. 615 p.

BONNER, J.T. 1952. Morphogenesis. An essay on development. Academic Press, Inc., New York. 296 p.

CONRAD, G.W., and R. RAPPAPORT. 1981. Mechanisms of cytokinesis in animal cells. *In* A.M. Zimmerman and S.A. Forer [eds.], Mitosis/cytokinesis. Academic Press, Inc., New York, pp. 365-396.

GOLDMAN, R., T. POLLARD and J. ROSENBAUM [eds.]. 1976. Cell motility. Cold Spr. Harbor Lab. 3 vols.

HYNES, R. O., and C.F. FOX [eds]. 1980. Tumor cell surfaces and malignancy. Progress in Clinical and Biological Research, Vol. 41. Alan R. Liss, Inc., New York. 961 p.

JACOBSON, C.-O., and T. EBENDAL [eds]. 1978. Formshaping movements in neurogenesis. Almqvist & Wiksell International, Stockholm. 257 p.

PIERCE, G.B., R. SHIKES, and L.M. FINK. 1978. Cancer: a problem of developmental biology. Prentice-Hall, Inc., Englewood Cliffs, N.J. 242 p.

PORTER, R., and D.W. FITZSIMONS [eds.]. 1978. Locomotion of tissue cells. Ciba Foundation Symposium 14 (new series). Elsevier North-Holland Publishing Company, Amsterdam. 381 p.

SATIR, P.R., and [illegible] 1985. [illegible] and evolution. Macmillan, New York, in press.

SUSSMAN, R., and C.W. WESSELLS [eds.]. 1980. The cell surface: mediator of developmental processes. Academic Press, Inc., New York. 374 p.

THOMPSON, D'ARCY W. 1942. On growth and form. Cambridge Univ. Press, Cambridge/Macmillan Publishing Co., Inc., New York. 1116 p.

TRINKAUS, J.P. 1976. On the mechanism of metazoan cell movements. *In* G. Poste and G.L. Nicolson [eds.], The cell surface in animal embryogenesis and development. Elsevier/North-Holland Publishing Company, Amsterdam, pp. 225–329.

VASILIEV, J. M., and I.M. GELFAND. 1981. Neoplastic and normal cells in culture. Cambridge Univ. Press, Cambridge. 372 p.

WEATHERBEE, J.A. 1981. Membranes and cell movement: interactions of membranes with the proteins of the cytoskeleton. Int. Rev. Cytol. Suppl. 12: 113–176.

WESSELLS, N.K. 1977. Tissue interactions and development. W.A. Benjamin, Inc., Menlo Park, Calif. 276 p.

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